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ENVIRONMENTAL FACTORS AFFECTING EFFICACY OF BIFENTHRIN-TREATED VEGETATION FOR MOSQUITO CONTROL

SANDRA A. ALLAN,[1] DANIEL L. KLINE,[1] AND TODD WALKER[2]

ABSTRACT. The use of pesticide-treated vegetation as a barrier for control of nuisance and disease-bearing mosquitoes has become an option for mosquito management for home owners and public health and mosquito control professionals. Potted wax myrtle and azalea plants were treated with bifenthrin (0.79% AI) at maximum label rate using backpack and electrostatic sprayers and exposed to various treatments that could affect the residual degradation of the applied pesticides. Treatments included leaf aspect, simulated rainfall, shade, and natural sun exposure with the residual effectiveness of leaves examined in tarsal contact Petri dish assays using laboratory-reared *Aedes aegypti.* There was no significant difference in efficacy between the adaxial (top) or abaxial (bottom) surfaces of electrostatically or backpack-treated leaves. Significant differences existed between application method, plant species, and exposure with most significant effects between weeks 1 and 4. Simulated heavy rainfalls applied 3 times weekly reduced knockdown by leaves treated with electrostatic and backpack methods with reductions seen as soon as 1 wk after treatment. Reductions were seen with both wax myrtle and azalea leaves and after 1, 4, and 24 h contact of mosquitoes to leaves. Placement of plants with full exposure to sunlight also significantly reduced efficacy compared to plants placed in the shade. Differences were observed most often for 4 and 24 h knockdown counts, and significant decreases were seen from week 4 onwards. Clearly factors such as rain and exposure to sun impact degradation of efficacy of bifenthrin-treated vegetation in the field. Degradation of bifenthrin efficacy was slowest in sites protected from rain and sun, which coincide with preferred resting site locations for many mosquito species.

KEY WORDS bifenthrin, mosquito control, *Aedes aegypti,* leaves, wax myrtle, azalea

INTRODUCTION

Activity of mosquitoes is regulated by a circadian periodicity with much of each day spent being inactive or resting. Resting mosquitoes, representative of all stages and ages, consist of those resting after emergence or appetitive and consummatory flights and are distributed among a variety of sites including substrates (e.g., soil, rocks), vegetation, human habitation, and animal shelters (Clements 1999). Although vegetation is a common resting site of mosquitoes, considerable differences exist between the species predominately collected (Service 1971, Karoji 1980, Irby and Apperson 1992, Burkett-Cardena et al. 2008) with the collection of some *Aedes* spp. strongly associated with vegetation (Mullen 1971, Irby and Apperson 1992, Burkett-Cardena et al. 2008). Additionally, the distribution of mosquitoes in vegetation appears localized (Service 1971, Karoji 1980) with higher densities reported in association with greater vegetative cover (Bidlingmayer 1971), decreased light intensity formed by the leaf cover, and the presence of particular plant species (Service 1971). Therefore the propensity for certain mosquito species to rest on vegetation provides an excellent opportunity for targeted control.

A wide range of pesticides have been evaluated for residual foliar applications for mosquito control and include DDT (Madden et al. 1947, Ludvik 1950), bendiocarb (Perich et al. 1993), fenitrothion (Hudson 1984), malathion (Taylor et al. 1975, Anderson et al. 1991, Perich et al. 1993), permethrin (Helson and Surgeoner 1983, Anderson et al. 1991, Perich et al. 1993, Cilek and Hallmon 2006), lambda cyhalothrin (Trout et al. 2007, Cilek and Hallmon 2008), beta-cyfluthrin (Cilek and Hallmon 2008), tau-fluvalinate (Cilek and Hallmon 2008), deltamethrin (Cilek and Hallmon 2006), and bifenthrin (Trout et al. 2007, Cilek 2008, Doyle et al. 2009). In recent field studies applications of bifenthrin on vegetation around residential sites or park areas provided control of host-seeking mosquitoes for 4–6 wk (Doyle 2007, Trout et al. 2007, Cilek 2008). Degradation of pesticides on foliage can be related to application methods, formulation, environmental factors, and plant characteristics (Ebeling 1963, Edwards 1975). The current study reported here was conducted in conjunction with a larger field study on efficacy of bifenthrin-treated barriers and addresses the effect of several factors that may impact the residual efficacy of residual treatments. To further understand the processes that affect degradation of the efficacy of bifenthrin application on foliage, we examined the role of exposure to sun, rainfall, and plant species. Additionally, comparisons were made of the aspect of leaf and the application method on the efficacy of the treatment during the course of the study.

[1] USDA/ARS, 1600 SW 23rd Drive, Gainesville, FL 32608.

[2] Naval Entomology Center of Excellence, Naval Air Station, PO Box 43, Bldg 937, Jacksonville, FL 32212-0043.

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MATERIALS AND METHODS

Plants

Trials were conducted using wax myrtle (*Myrica cerifera* (L.)) (1.4–1.7 m tall), which is a common native plant used in natural landscaping and previously reported as a resting plant for mosquitoes (Bidlingmayer 1971). Additionally, azaleas (*Rhododendron simsii* "Southern pink") (0.4–0.5 m tall), which are common landscaping plants in residential and park areas, were included in the study (Cilek 2008). Pretreatment assays were conducted with leaves from these plants for each trial to verify that no residual insecticide was present before each treatment. No mortality was noted in these assays (data not presented). Leaves for each assay were collected by randomly selecting leaves over the plant and placing them in a prelabeled resealable plastic bag. About 10–15 leaves were used for each Petri dish. Care was taken to avoid leaves from new growth that occurred after insecticide treatment. Leaves were sampled 1–2 days before treatment, on the day of treatment, and at weeks 1, 2, 4, 6, and 7. All plants were watered daily with drip irrigation.

Pesticide application

Pesticide was applied by using either a backpack mist blower (model SR-420, Andrea Stihl, Waiblingen DL, Germany) or an electrostatic applicator (Electrolon BP-2.5, Electrostatic Sprayer, Watkinsville, GA) using a similar volume of active ingredient (AI). Pesticide application was made by a certified pesticide applicator until pesticide runoff following label instructions for TalstarOne® (79.01 ml AI/liter) (maximum label rate). Application was made with mist directed toward the vegetation from 0.25 to 2 m above the ground. Plants were placed in rows with ca. 0.5 m between plants and ca. 1.5 m between rows. During spraying there was little to no wind and no rainfall in the first 24 h. Efforts were made to thoroughly spray all plants so that application of treatment was even as possible. Two trials were conducted, with the first pesticide application on 18 June 2008 and the second on 6 August 2008. The volume of finished spray applied by the Stihl SR42 and the Electrolon BP 2.5 for both trials was 3.6 gal (13.6 liters) and 4.0 gal (15.1 liters), respectively. Control plants remained untreated. After pesticide treatment, plants were randomly selected and allocated to different trials.

Assays

Laboratory Petri dish assays that forced tarsal contact of mosquitoes with treated leaves were conducted to evaluate efficacy of insecticide residues on leaves (Doyle et al. 2009). For each test, leaves were collected and tested on the day of sampling or frozen at -20°C in heavy resealable plastic bags and tested later. Previous data indicated that freezing did not alter efficacy of treated leaves (Doyle et al. 2009). Leaves were removed from the freezer, laid on paper towels, and allowed to air dry before being placed in Petri dishes. Care was taken not to rub the surface of the leaves. For each test, leaves were fastened to double-stick tape that covered the entire base of a Petri dish (100 mm diameter). Portions of leaves excessive for covering the bottom of the Petri dish were cut with a razor blade and removed so that each Petri dish provided the same amount of surface area of foliage. Care was taken to cover exposed tape with cotton fibers from a cotton ball so that mosquitoes did not become stuck. Unless otherwise specified, all leaves were tested with top (adaxial) surface facing upwards. Gloves were worn when handling leaves and changed between treatments. During these tests it was observed that mosquitoes rested only on the vegetation, not on the plastic sides or lids of the dishes; thus, each mosquito was equivalently exposed.

For each assay, ten 7–10-day-old laboratory-reared pyrethroid-susceptible female *Aedes aegypti* were used (Pridgeon et al. 2008). After 1, 4, and 24 h of exposure to treated leaves, mosquitoes were examined for knockdown. A mosquito was considered knocked down if it was unable to stand after the dish was tapped. Each assay was replicated 5 times for each of the 2 trials and data combined for analysis. Petri dishes were stored at 70–85% relative humidity and 20–22°C during the tests. Control assays were conducted using untreated leaves and were conducted at each test date. If control mortality occurred, it was used to correct treatment mortality following Abbott's formula (Abbott 1925). Data are presented as the percentage of the total number of mosquitoes in a Petri dish that were knocked down.

Effect of leaf aspect

To evaluate whether there was a difference in the residual effectiveness of insecticide on the top (adaxial) or bottom (abaxial) surface of leaves, leaves of wax myrtle were collected and fastened with either adaxial or abaxial surfaces upward for contact with tarsi of mosquitoes. Untreated wax myrtle plants were used for controls. Treated and control plants for this study were maintained with full sun exposure and natural rainfall.

Effect of sun and shade

To evaluate the influence of natural exposure to sun on residual effectiveness, 2 wax myrtle plants and 2 azalea plants were placed both with full exposure to sunlight and under a dense canopy of tree with no direct exposure to sunlight. One untreated wax myrtle and azalea

plant was placed in the sun and in the shade to serve as controls. Light intensities recorded with a light meter (model 401036 [400–700 nm], Extech Instruments, Waltham, MA) indicated an average light intensity in the shade that was 0.08% of that in the sun (77.8 lux in shade, 91,170 lux in sun). Plants in full sun received natural rainfall, and those in the shade received an average of 28% less rainfall.

Effect of rainfall (simulated rainfall)

The influence of rainfall on efficacy on degradation of bifenthrin foliar residues was evaluated by providing simulated rainfall to plants in a greenhouse. Wax myrtle and azalea plants that were treated with either electrostatic or backpack sprayers were divided into 2 groups: those with no rainfall and those with heavy rainfall characteristic for June–September in north central Florida. Water was sprayed over individual plants 3 times a week (24 cm total). Control plants were untreated wax myrtles and azaleas maintained in a greenhouse on drip irrigation. Plants were held in a greenhouse to prevent the confounding influence of natural rainfall but to provide natural light conditions. However, light intensity was reduced through lighthouse walls and screening to an average of 21.6% of the light intensity of ambient light (19,690 lux).

Effect of plant species

Comparisons were made between wax myrtle and azalea leaves for the rain exposure, sun, and shade studies.

Weather records

Records for rainfall, temperature, and sunlight intensity were obtained from the University of Florida Department of Physics weather station (2.4 km from plants).

Statistical analysis

Data were arcsine-transformed and untransformed means are presented in figures. To compare the effect of sun exposure or rain on backpack- and electrostatically treated leaves, paired t-tests with unequal variance were used to compare means ($P = 0.05$). Data from the sun/shade and rain/no rain studies were tested using a 3-way ANOVA (PROC GLM, SAS Institute 1999) with interactive effects for plant (wax myrtle, azalea) by exposure (rain/no rain, sun/shade), application (backpack, electrostatic), by exposure, plant by application, and plant by application by exposure. Plant species, application method, and exposure (sun/shade, rain/no rain) were fixed effects in the model.

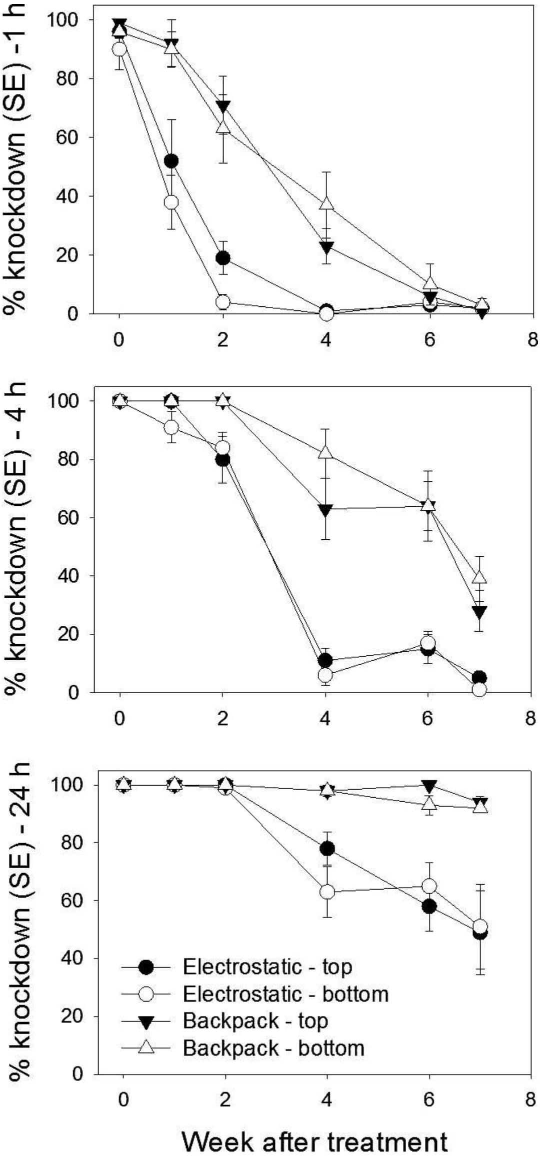

Fig. 1. Effect of leaf aspect on mosquito knockdown.

RESULTS

Effect of leaf aspect

In general, there was no difference in knockdown from the top or bottom of leaves for both backpack and electrostatic treatments (Fig. 1). After 1 h of exposure to treated leaves, knockdown did not differ between the top and bottom of the wax myrtle leaves for both electrostatic and backpack spraying at each week tested ($P > 0.05$) except for week 2, when knockdown with electrostatically treated leaves was considerably lower. At weeks 2 and 4, knockdown was greater at 1 h on leaves treated with the backpack sprayer compared with the electrostatic sprayer ($P <$

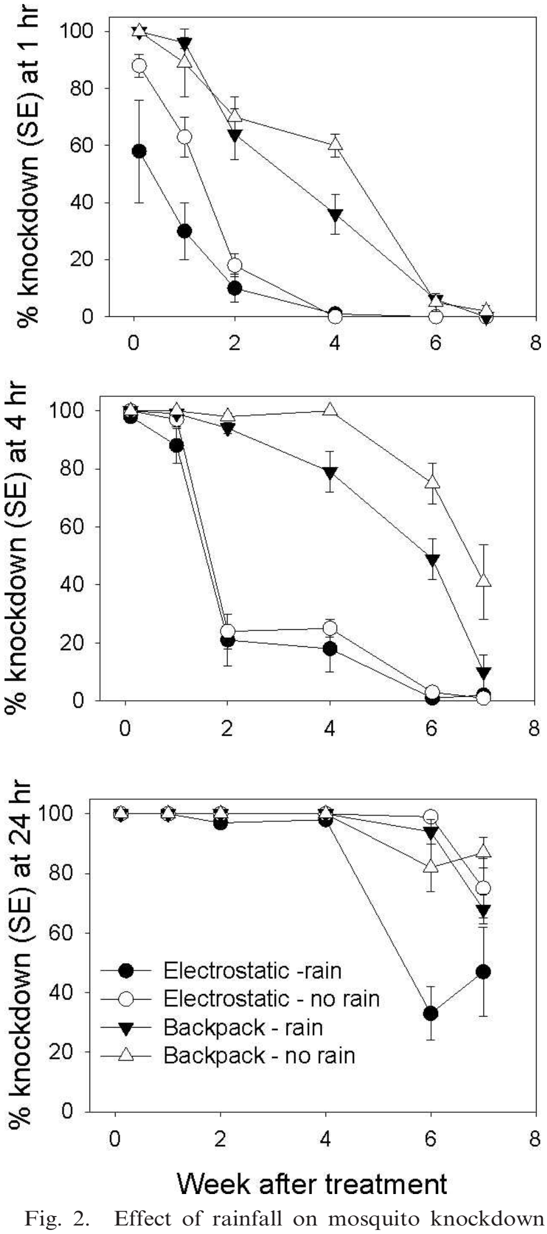

Fig. 2. Effect of rainfall on mosquito knockdown on wax myrtle leaves.

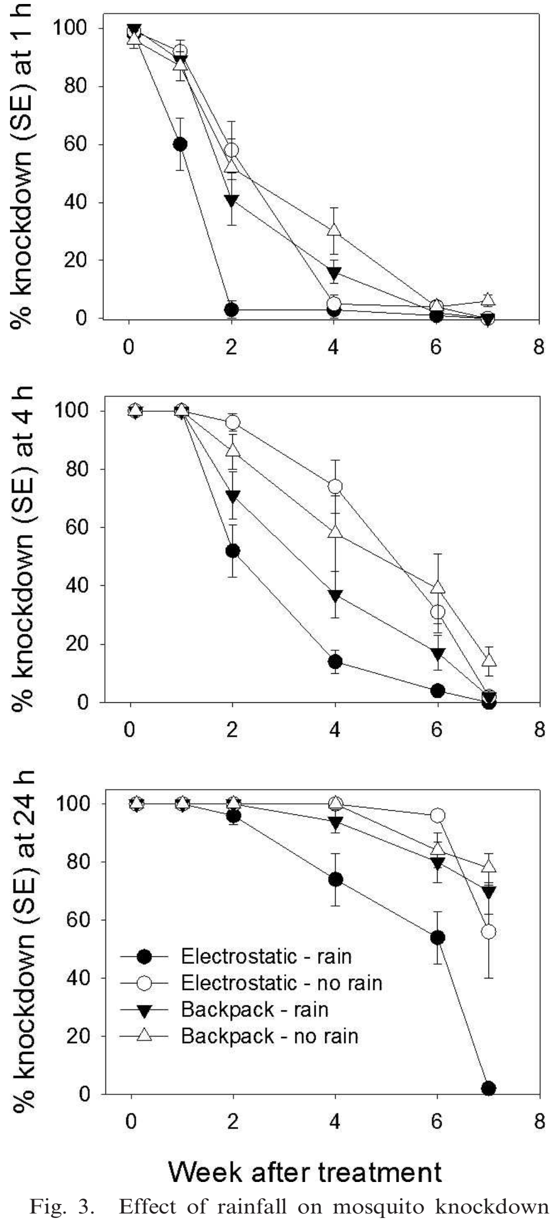

Fig. 3. Effect of rainfall on mosquito knockdown on azalea leaves.

0.05); however, by week 6, these differences had disappeared. After 4 h of exposure to leaves, there were no differences between knockdown after exposure to the top or bottom of the leaves within application ($P > 0.05$). Knockdown from the backpack sprayer was significantly greater (about 20–70%) than from the electrostatic sprayer at weeks 2, 4, 6, and 7 regardless of leaf surface ($P < 0.05$). After 24 h exposure to leaves, there was no difference between the top and bottom of the leaves for backpack-treated leaves regardless of application method ($P > 0.05$). However, at weeks 4, 6, and 7, exposure to the backpack-treated leaves elicited more knockdown than the electrostatically treated leaves ($P < 0.05$).

Effect of simulated rainfall

Simulated rainfall significantly decreased knockdown of both backpack and electrostatically treated wax myrtle and azalea leaves (Figs. 2 and 3). For wax myrtle leaves, after 1 h of exposure, knockdown was lower on rain-treated leaves compared with nonexposed leaves for weeks 1 and 2 for electrostatic applications and for backpack-treated leaves at week 4 ($P < 0.05$)

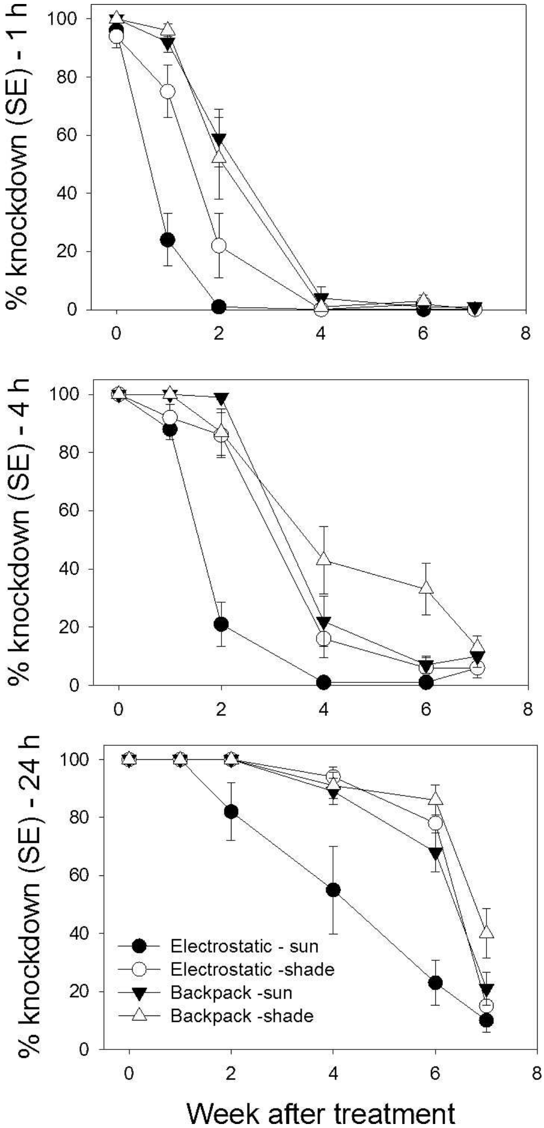

Fig. 4. Effect of full sunlight exposure on mosquito knockdown on wax myrtle leaves.

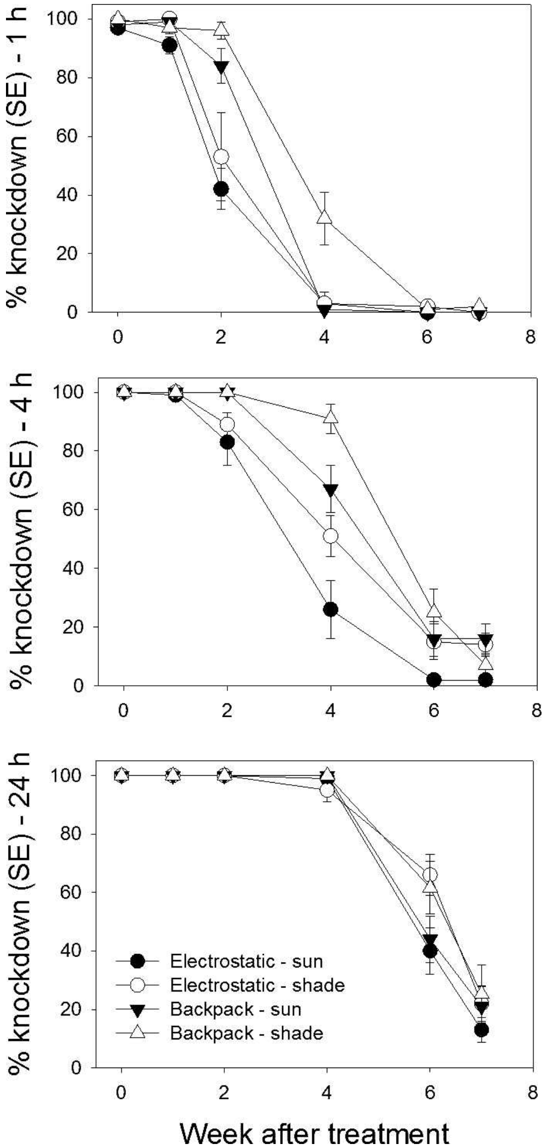

Fig. 5. Effect of full sunlight exposure on knockdown of mosquitoes on azalea leaves.

(Fig. 2). After 4 h of exposure, there was no difference in knockdown on electrostatically treated leaves exposed to rain regardless of week ($P > 0.05$). Rain-treated wax myrtle leaves were less effective for backpack-treated leaves at 4, 6, and 7 weeks ($P < 0.05$). By 24 h of exposure to treated leaves, leaves with no exposure to rain were more effective for both application methods at weeks 6 and 7 ($P < 0.05$).

After 1 h of exposure to rain-treated azalea leaves, efficacy of electrostatic applications was decreased at weeks 1 and 2, and efficacy of backpack applications decreased at weeks 4 and 7 ($P < 0.05$) (Fig. 3). After 4 h of exposure, similar decreases were seen at weeks 2 through 7 for both treatments and at week 7 for the backpack treatment ($P < 0.05$). After 24 h of exposure, knockdown on rain-exposed leaves were significantly less effective than unexposed leaves for the electrostatic treatments at weeks 4, 6, and 7 ($P >$ 0.05).

Effect of full sunlight exposure

Placement of plants in full sunlight resulted in reduced knockdown from backpack-treated and electrostatically treated wax myrtle and azalea leaves compared with leaves from plants that were placed in the sun (Figs. 4 and 5). For wax myrtle leaves, after 1 h of exposure, knockdown

Table 1. *F*-statistics from 3-way ANOVA on mosquito knockdown showing the effect of plant species, treatment exposure, and application methods (df = 1, 72) for the simulated rain and sun exposure study over 7 wk.

	Week					
Source	0.1	1	2	4	6	7
Effect of simulated rain						
Plant	2.47	75.08**	152.10**	10.56**	0.71	0.67
Exposure	2.33	13.06**	42.34**	4.69*	2.69	0.05
Application	2.56	68.37**	152.10 **	48.27**	30.90**	6.03*
Plant × exposure	2.33	1.30	1.60	0.81	0.08	10.72**
Exposure × application	1.75	0.02	0.40	2.94	2.43	4.43*
Plant × application	2.56	25.01**	122.5**	28.34**	16.32**	9.97**
Plant × application × exposure	1.75	5.42	0.40	17.93**	14.43**	2.68
Effect of sun exposure						
Plant	0.53	10.32**	21.33**	82.63**	1.79	1.00
Exposure	0.13	0.70	12.18**	7.34**	6.83*	3.59
Application	2.73	12.36**	40.05**	17.60**	10.84**	7.90**
Plant × exposure	1.18	0.25	7.73**	2.48	0.16	1.87
Exposure × application	0.13	0.70	24.10**	0.00	0.08	0.12
Plant × application	2.10	10.12**	9.10**	8.32**	0.85	2.71
Plant × application × exposure	0.13	0.25	17.63**	0.01	0.51	9.36**

* F-statistic significant at $P < 0.05$.
** F-statistic significant at $P < 0.01$.

was significantly lower at 1 and 2 wk to sun-exposed compared with shade-exposed electrostatically treated leaves ($P < 0.05$) (Fig. 4). After 4 h of exposure to leaves, efficacy of electrostatically leaves that were sun-exposed was lower than those held in the shade at weeks 2 and 4 ($P < 0.05$). There was no difference between backpack-treated leaves except at week 6 ($P > 0.05$). After 24 h of full sun exposure, treated leaves were significantly less effective than shaded leaves for electrostatic treatments for weeks 2, 4, and 6. There were no knockdown differences between sun or shade exposed backpack-treated leaves ($P > 0.05$). Responses of backpack-treated leaves were greater than electrostatically treated leaves only for a 1 h exposure at weeks 1 and 2 ($P < 0.05$).

No effect of sun treatment on azalea leaves was observed after 1 h of exposure ($P > 0.05$) except for a reduction in response to backpack leaves at week 2 and 4. After 4 h of exposure, knockdown from backpack-treated leaves that had been exposed to full sun had significantly decreased at week 4 and for electrostatically treated leaves at week 4 and 6. After 24 h of exposure, there were no differences between sun- or shade-exposed ($P > 0.05$). Differences between electrostatically treated and backpack-treated leaves were observed only after 1 h of exposure at week 4 for backpack-treated leaves and after 4 h of exposure at week 4 ($P < 0.05$).

Effect of plant species

Significant knockdown differences between plant species were present from weeks 1–4 for the simulated rain study and sunlight exposure study (Table 1). Significant differences also were present between exposures with differences between simulated rain and no rain from weeks 1–4 and differences between sunlight and shade exposure from weeks 2–6 in the sunlight exposure study. Application method was significant for weeks 1–7 in both studies. Interactions of plant and exposure, as well as exposure and application, were significant for week 7 of the simulated rain study and for week 2 for the sunlight study. An interaction of plant and application method was significant for weeks 1–7 in the simulated rain study and for weeks 1–4 in the sunlight study. Interactions between plant, application method, and sunlight exposure was significant in the sunlight study on weeks 2 and 7. Interactions between plant, application method, and simulated rain exposure were significant for weeks 4 and 6 (Table 1).

Weather parameters

There was no difference between rainfall in trial 1 (19.6 ± 8.4 cm/wk) and trial 2 (27.7 ± 4.3 cm/wk) ($t = 0.38$, df = 8, $P = 0.35$). Average daily temperatures during trials 1 and 2 were 26.7 and 26.2°C, respectively.

DISCUSSION

The rate of disappearance of pesticide residues from treated vegetation is affected by factors associated with physical characteristics of the plant, formulation of pesticides, degree of tenacity of deposits, and factors causing erosion of the surface deposits (rain, wind, mechanical removal)

(Ebeling 1963, McDowell et al. 1984, Willis et al. 1984). In addition, factors relating to the nature of the pesticide such as volatilization, microbial, and chemical decomposition (sun, ultraviolet) are important (Ebeling 1963, Edwards 1975, Willis and McDowell 1987).

We found that exposure to heavy rainfall clearly decreased the efficacy of bifethrin-treated leaves over time. Previously Perich et al. (1993) compared simulated rainfall spanning from 0.5 to 2 cm every 2 days with no rainfall and their effects on permethrin, carbamate, and malathion residues on a range of plant types. There were no differences in adult mosquito mortality with the permethrin treatment, but mortality was reduced with bendiocarb and malathion treatments. Although the simulated rainfall in our study was considered heavy (25.4 cm) for north central Florida, it was representative of the normal local seasonal rainfalls and with the average rainfall during trial 1 and 2 being 19.6 cm/wk and 27.68 cm/wk, respectively. Decreases in bifenthrin residue efficacy observed in our study within 1 wk after treatment. At some intervals (i.e., azalea leaves treated by electrostatic sprayer at week 1 with 1 h exposure), there was as much as a 10-fold difference in knockdown between rain- and no-rain-exposed leaves. This loss of efficacy as a direct result of rainfall is consistent with other results observed with bifenthrin (Mulrooney and Elmore 2000) and other pesticides (McDowell et al. 1984).

Bifenthrin is not very water soluble (Meister and Sine 1997), nor is it absorbed in plant foliage or translocated in the plant (US EPA 1988). Degradation of the efficacy of bifenthrin-treated leaves is likely due to the erosion of surface deposits that are characteristically affected by rain (Ebeling 1963, Edwards 1975). Apart from mechanical abrasion, the main cause of wax erosion is rain. The effect of the impact of the rain droplet is over many wax crystals with relatively low regeneration of wax (Neinhuis and Barthlott 1997). Even minimal amounts of simulated rain erode epicuticular wax from leaves that may, in turn, result in loss of pesticide deposits (Baker and Hunt 1986).

Bifenthrin is relatively photostable compared with other pyrethroids (Mokrey and Hoagland 1989). In our study plants exposed to full sunlight clearly showed more rapid loss of efficacy than those held under shade conditions. Plants held in the sun were exposed to natural rainfall, and those held in the shade received about 28% less rainfall than those in the sun due to the protection from the leaf canopy. It is possible that the loss in efficacy under sun conditions may have resulted from the greater exposure to rainfall compared with those in the shade. In any event, treated leaves in shade conditions retained efficacy longer than those under full sun exposure.

The lack of difference in bifenthrin efficacy between the tops or bottoms of leaves was likely due to similar exposure to sunlight and rainfall due to the structure of the wax myrtle leaf. However, under operational conditions, pesticide application to the top and bottom of leaves may be less even with the bottoms receiving the lower deposits (Womac et al. 1993). Additionally, plant species may differ in waxiness between upper and lower surfaces of leaves (Neinhuis and Barthlott 1997, Muller and Reiderer 2005), potentially affecting wetting properties and pesticide deposition (Ebeling 1963). In an experimental study on rainfastness of bean leaves, Pielou and Williams (1961) reported that the amount of rain required to remove residues differed between the top and bottom of leaves with the bottom of leaves retaining pesticide the most residue. The persistence of a particular pesticide depends greatly on the method of application (Edwards 1975). Air-assisted electrostatic sprayers improve deposition of pesticide droplets into the plant canopy, into the middle of plants, and on the underside of cotton leaves (Sumner et al. 1984) but may be more prone to erosion of efficacy, particularly with rain.

It is difficult to predict the duration of contact that a mosquito would have with treated vegetation because resting may be transitional (to a more optimal site) or for the entire nonactive portion of the day. For this reason it is difficult to predict the duration of contact and subsequently the dose of pesticide that a mosquito would contact. In this study efficacy data for each test were obtained at 3 time intervals (1, 4, and 24 h) after exposure to better assess the impact of the treatments (sun, shade, simulated rain) on efficacy over time. Contact exposure for 24 h likely overrepresents the mortality under natural conditions, and the 1 or 4 h exposure times are likely more representative of the normal duration of contact between a resting mosquito and treated vegetation. Additionally, the effects of sun and rain exposure on leaves were observed more clearly with shorter durations of contact between mosquitoes and leaves. Contact exposure for 24 h does, however, provide a comparison for mortality as noted in previous studies (Perich et al. 1993, Cilek and Hallmon 2006, Doyle 2007, Trout 2007, Cilek 2008). Efficacy of bifenthrin-treated leaves in our bioassays was in agreement of other studies of >70% mortality for at least 4 wk (Trout et al. 2006, Cilek 2008, Doyle et al. 2009).

The application of bifenthrin to barrier vegetation is a promising approach for targeted control of resting mosquitoes. The slower degradation of pesticide on leaves protected from rainfall and shade enhances the duration of effective treatment in the darker areas with denser vegetation preferred as resting sites of mosquitoes. Therefore we recommend that if residual pesticides are applied to vegetation for the purpose of providing

adult mosquito control, they should be applied to dense, sheltered vegetation.

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